

## Morphology of the epididymal region and ductus deferens of the turkey (*Meleagris gallopavo*)\*

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### INTRODUCTION

The anatomy and physiology of the reproductive tract of the chicken have been extensively studied by Gray (1937), Parker, McKenzie & Kempster (1942), Lake (1957) and Tingari (1971). The excurrent duct system of the chicken testis consists of an epididymal region adjacent to the testis and a long convoluted ductus deferens which terminates in an enlarged ejaculatory duct. Unlike mammals, the chicken does not have accessory sex glands which add secretory products to the semen, but does have secretory cells in the epithelium of the excurrent ducts (Tingari & Lake, 1972).

The use of artificial insemination in the commercial turkey industry has established the need for a thorough understanding of the anatomy and physiology of semen production and transport in the domestic turkey (Lorenz, 1970). However, studies of the structure and function of the male reproductive tract of the turkey have not been extensively reported.

It was therefore decided to study the morphology of the turkey epididymal region and ductus deferens so as to establish a norm for future physiological and pathological investigations of these tissues.

### MATERIALS AND METHODS

Eight yearling white turkey breeder males were selected as producers of normal white coloured semen based upon the criteria described by Thurston *et al.* (1975). The turkeys were anaesthetized with sodium pentobarbitol and killed by phlebotomy. Abdominal organs were removed to expose the testes and associated ducts.

Fixative (3% glutaraldehyde in Millonig's (1961) phosphate buffer at pH 7.35 and 22 °C) was poured over the reproductive tract before further dissection. The testis was then injected with fixative in several places beneath the tunica albuginea, using the procedure described by Dal Lago & Lucke (1973). After removal from the body cavity, the testis, epididymal region and ductus deferens were placed in 3% buffered glutaraldehyde at 4 °C and cut into 1 mm<sup>3</sup> pieces. Larger areas of these tissues were fixed in glutaraldehyde at 22 °C for two days, embedded in paraffin and

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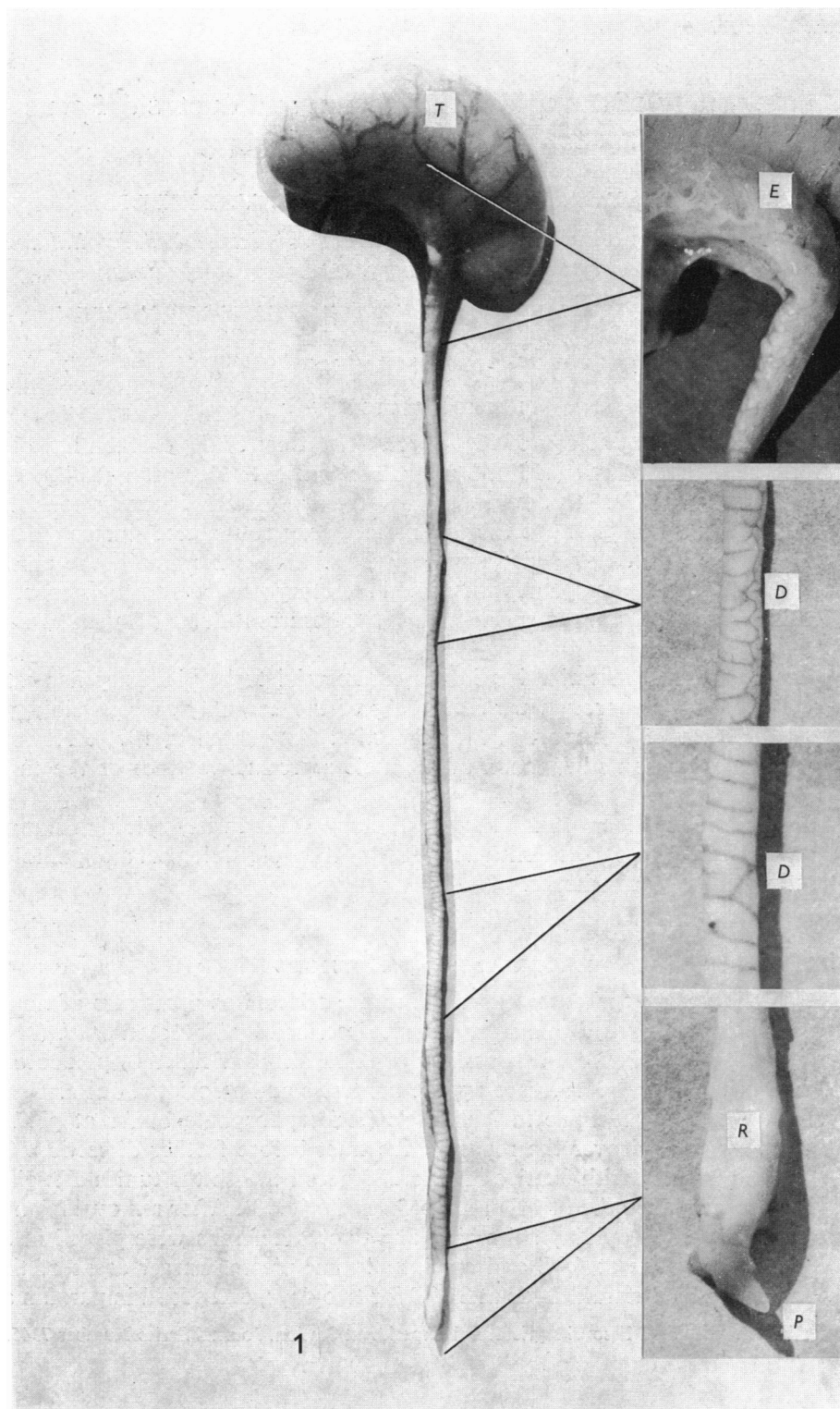


Fig. 1. Gross anatomical structure of the male turkey reproductive tract. (T), testis; (E), epididymal region; (D), ductus deferens; (R), receptaculum; (P), papilla.

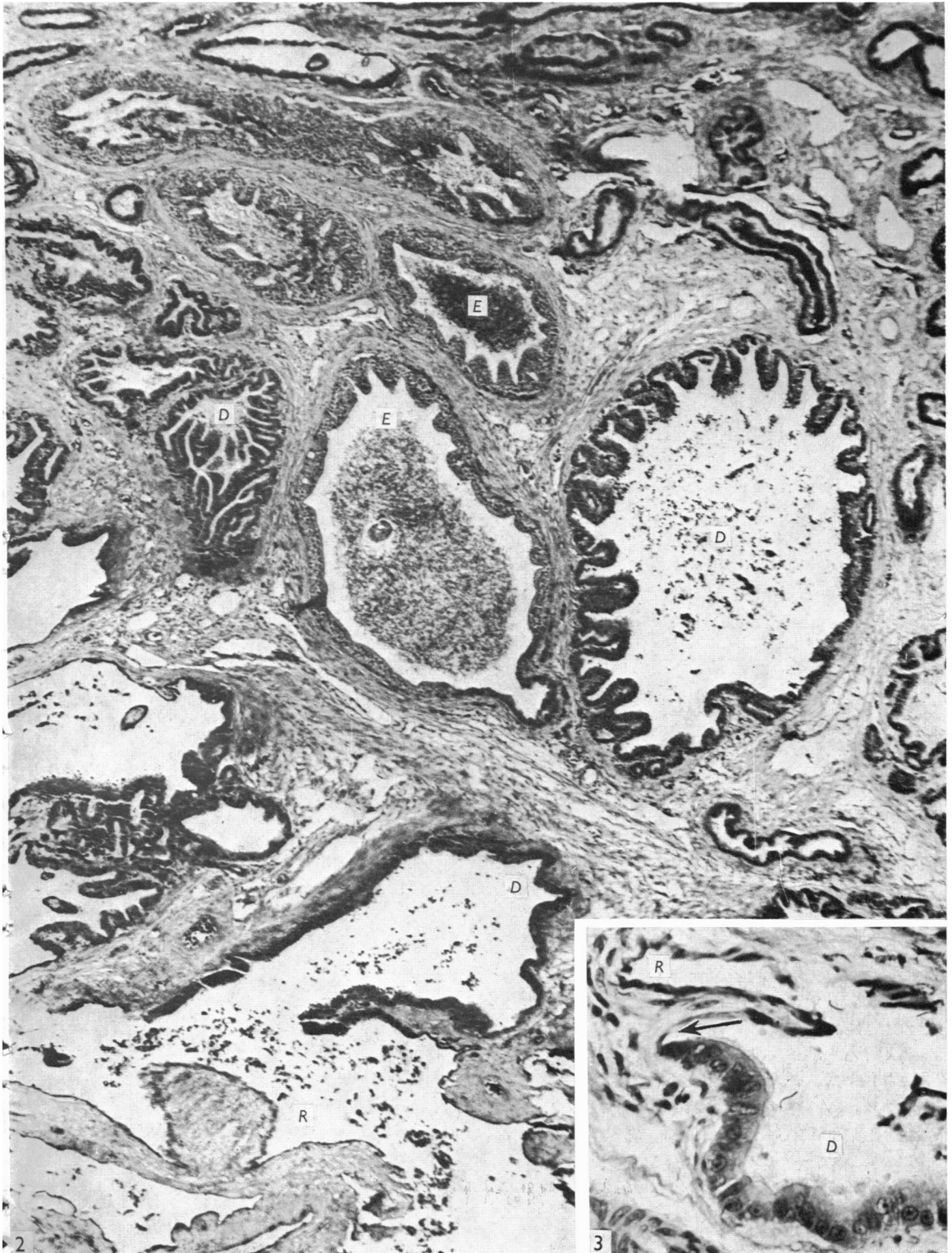
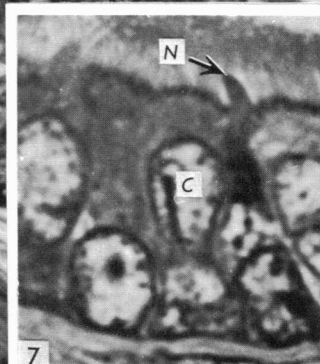
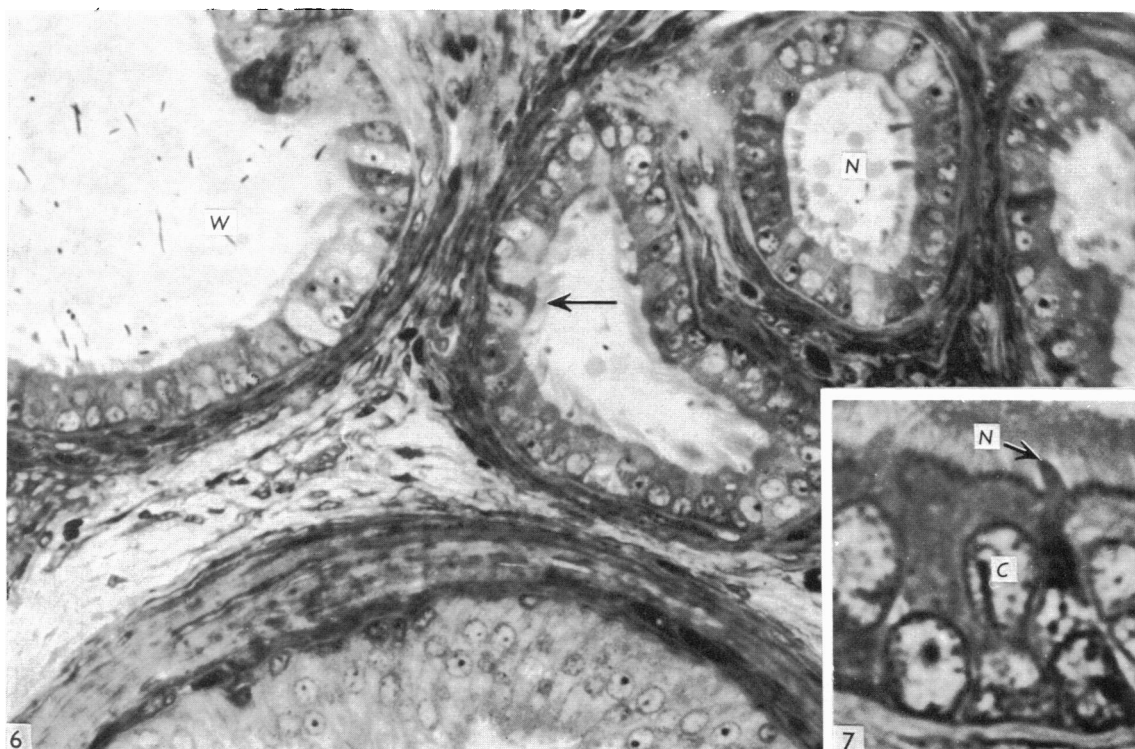
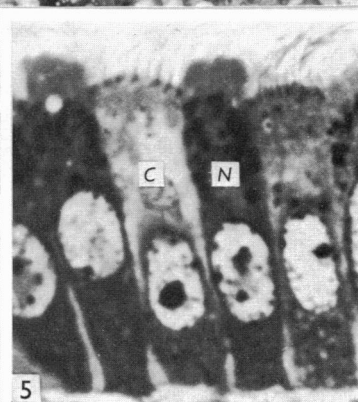
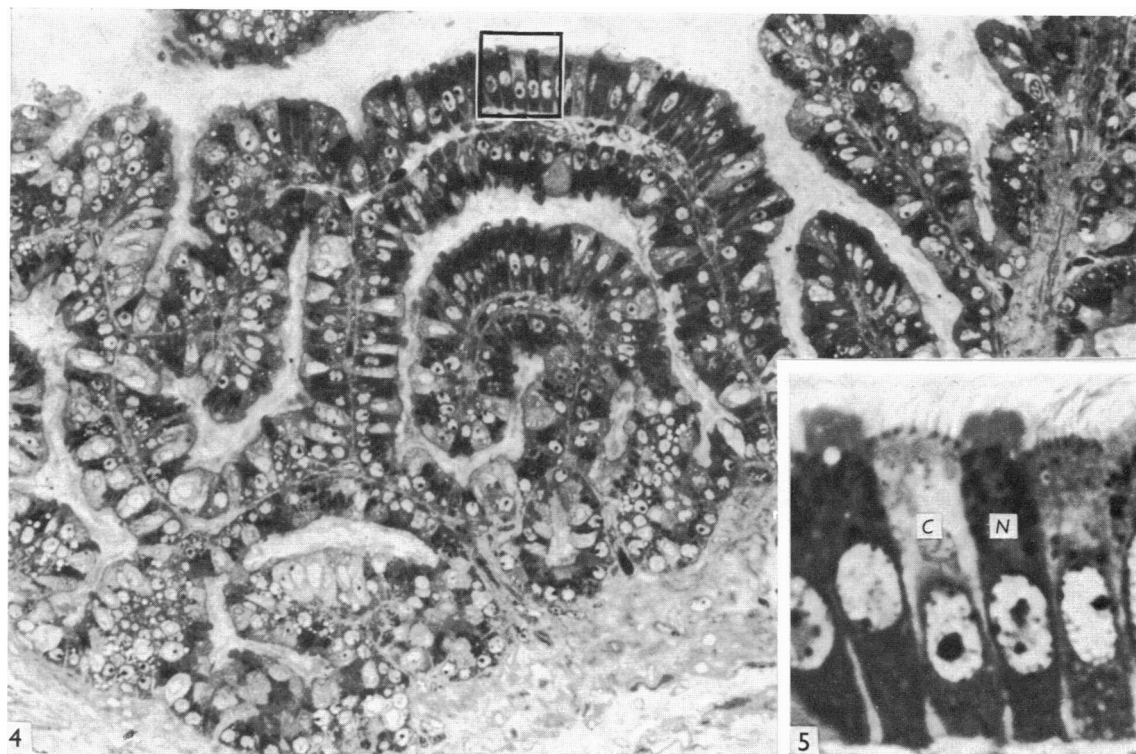


Fig. 2. The epididymal region. Cross sections of the ductus epididymidis (E) are in close proximity to the rete testis (R) and ductuli efferentes (D). Haematoxylin and eosin.  $\times 68$ .

Fig. 3. Squamous cells of the rete testis epithelium (R) abruptly change (arrow) into columnar cells at the origin of the ductuli efferentes (D). Haematoxylin and eosin.  $\times 450$ .



cut at 5  $\mu\text{m}$  for routine haematoxylin and eosin staining and light microscopic examination.

The smaller 1 mm<sup>3</sup> pieces of tissue were fixed at 4 °C for one hour, rinsed in Millonig's (1961) phosphate buffer, and post-fixed for 1 hour at 4 °C in 2 % osmium tetroxide, phosphate buffered at pH 7.35. Following a buffer rinse, the samples were dehydrated in a graded series of ethanols, cleared in propylene oxide and embedded in Epon 812 (Luft, 1961). Thin 1  $\mu\text{m}$  sections of the Epon embedded tissues were cut with a Sorvall MT2-B ultramicrotome, and stained with toluidine blue and basic fuchsin.

## RESULTS

### *Morphological features*

The normal turkey testis (Fig. 1) was large (6–7 cm in length), soft to touch and with gross characteristics similar to those described for the chicken testis (Tingari, 1971). Lying dorsomedial to the testis was the white epididymal region approximately 3 cm in length (Fig. 1). This region contained the rete testis, ductuli efferentes, connecting ductules and ductus epididymidis. The rete testis discharged into the ductuli efferentes which terminated at the beginning of the connecting ductules. The connecting ductules anastomosed in several places with the ductus epididymidis, which continued caudally as the highly convoluted ansae ductus deferentis. The ductus deferens (Fig. 1) was approximately 20 cm long, and gradually increased in diameter as it passed caudally to the cloaca. A sac-like receptaculum comprised the terminal portion of the ductus deferens and the lumen of this organ communicated with the lumen of the urodeum through a papilla.

*Rete testis.* The rete consisted of thin-walled channels for conveying spermatozoa from the openings of the seminiferous tubules of the testis (Fig. 2). The rete epithelium consisted of squamous or low cuboidal cells (1.5–12  $\mu\text{m}$  in height) which changed abruptly into columnar cells at the beginning of the ductuli efferentes (Fig. 3).

*Ductuli efferentes testis.* The ductuli efferentes were large ducts, approximately 800  $\mu\text{m}$  in diameter. Their epithelial lining was highly convoluted and consisted of pseudostratified columnar cells 20–25  $\mu\text{m}$  in height which were attached to the basement membrane (Fig. 4). Ciliated and non-ciliated columnar cells were in the majority, but occasional basal cells were also present. Ciliated cells were light

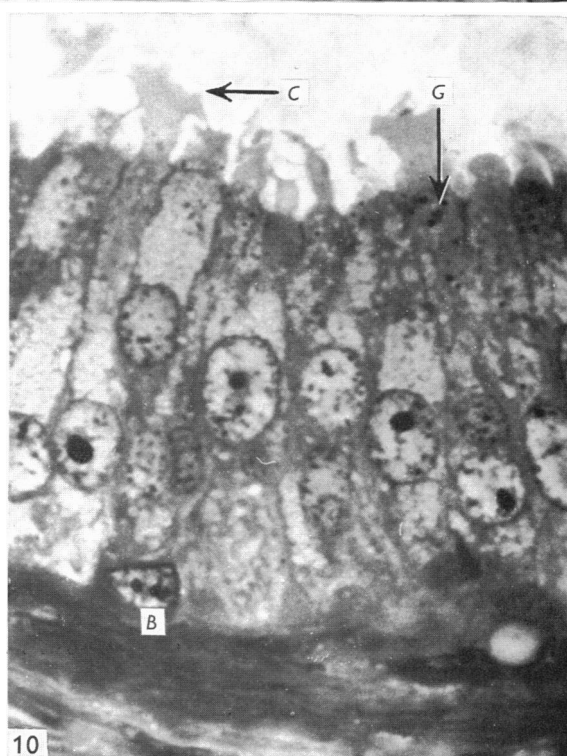
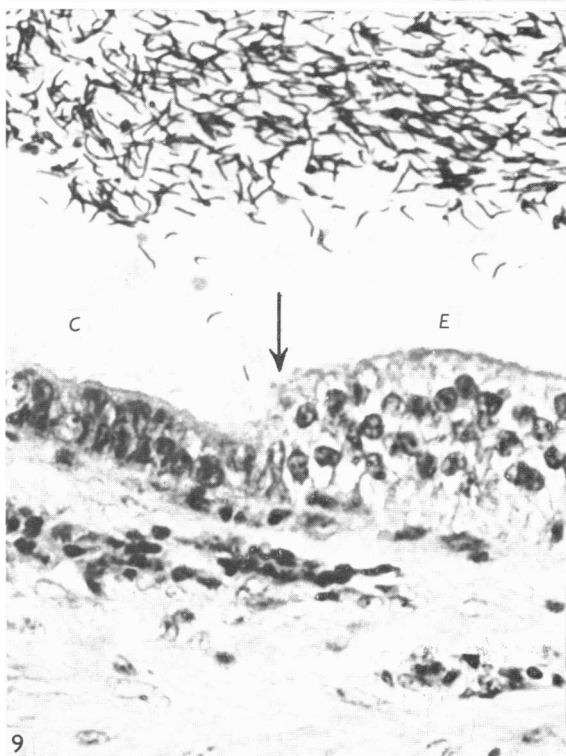
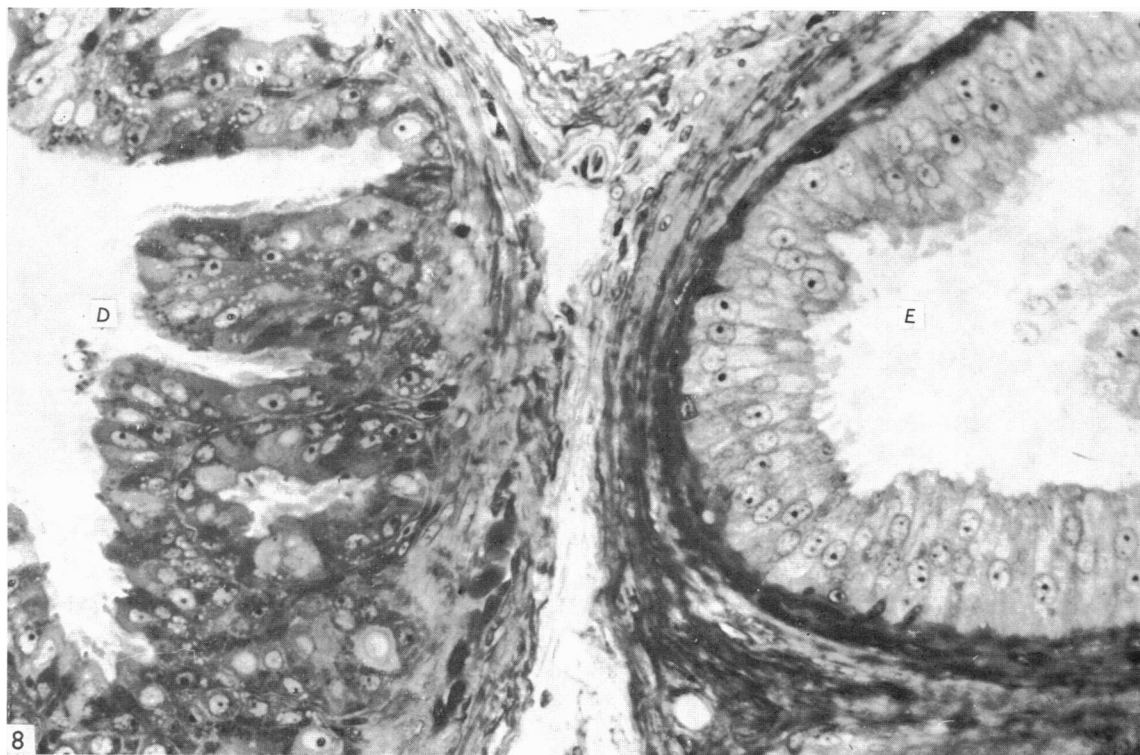
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Fig. 4. Ductuli efferentes showing the highly folded columnar epithelium which characterizes these ductules. Toluidine blue and basic fuchsin.  $\times 345$ .

Fig. 5. Higher magnification of non-ciliated (N) and ciliated (C) cells of the ductuli efferentes shown in Fig. 4. Note the dark granules in the apical cytoplasm of the non-ciliated cells and the basal bodies at the apex of the ciliated cells.  $\times 1840$ .

Fig. 6. Wide (W) and narrow (N) connecting ductules are distinguished by their smooth epithelial contour and abundance of ciliated cells (arrow). Toluidine blue and basic fuchsin.  $\times 550$ .

Fig. 7. Non-ciliated (N) and ciliated (C) cells of a narrow connecting ductule. The non-ciliated cells are few in number, and are characterized by the presence of chromatophilic granules and cytoplasmic blebbing. Toluidine blue and basic fuchsin.  $\times 2200$ .



staining and contained elongated nuclei, while the non-ciliated cells were dark staining with spherical nuclei. Non-ciliated cells showed a few vacuoles in their basal cytoplasm and dense chromatophilic granules in their apical cytoplasm (Fig. 5).

*Connecting ductules.* Narrow and wide connecting ductules (Fig. 6) were found in the epididymal region. The epithelial surface of these tubules had a smooth contour which was lined by pseudostratified columnar cells 12–14  $\mu\text{m}$  in height. Ciliated cells were abundant and occasional non-ciliated cells were also found. The nuclei of the ciliated cells lay in the apical as well as in the basal portion of the cells (Fig. 7). The non-ciliated cells were identified by their basal nuclei, dark staining cytoplasmic granules, and slight cytoplasmic blebbing of the cells into the ductal lumen (Fig. 7). Ciliated cells were more frequently found in the connecting ductule epithelium than in the epithelium of the ductuli efferentes (Fig. 8). Although evidence was not conclusive, the narrow ductules appeared to join the wide ductules which changed abruptly into the ductus epididymidis (Fig. 9).

*Ductus epididymidis.* The ductus epididymidis appeared highly tortuous and was not confined to the outer border of the epididymal region. Sections of the duct were found peripherally as well as in close proximity to the rete testis (Fig. 2). The mucosa was not as convoluted as that of the ductuli efferentes (Fig. 8) and was pseudostratified with non-ciliated columnar cells (approximately 26  $\mu\text{m}$  in height) and occasional basal cells. The non-ciliated cells all stained with approximately the same intensity and their nuclei were similar in size. The smaller basal cells were located next to the basement membrane; they stained intensely and their nuclei occupied most of the cytoplasm. Numerous bleb-like cytoplasmic extensions of the columnar cells projected from the epithelium into the lumen (Fig. 10).

*Ductus deferens.* The largest segment of the ductus deferens was highly sinuous and appeared morphologically similar to the ductus epididymidis (Fig. 11). The ductus was lined with a pseudostratified columnar epithelium, the distal section containing increased numbers of basal cells. The ductus deferens had a thick muscular layer and a wider lumen caudally (3 mm diameter) than cranially (0.3 mm diameter). The columnar epithelial cells had numerous bleb-like projections which extended into the lumen (Fig. 12). Many blood capillaries lay close to the epithelial basement membrane.

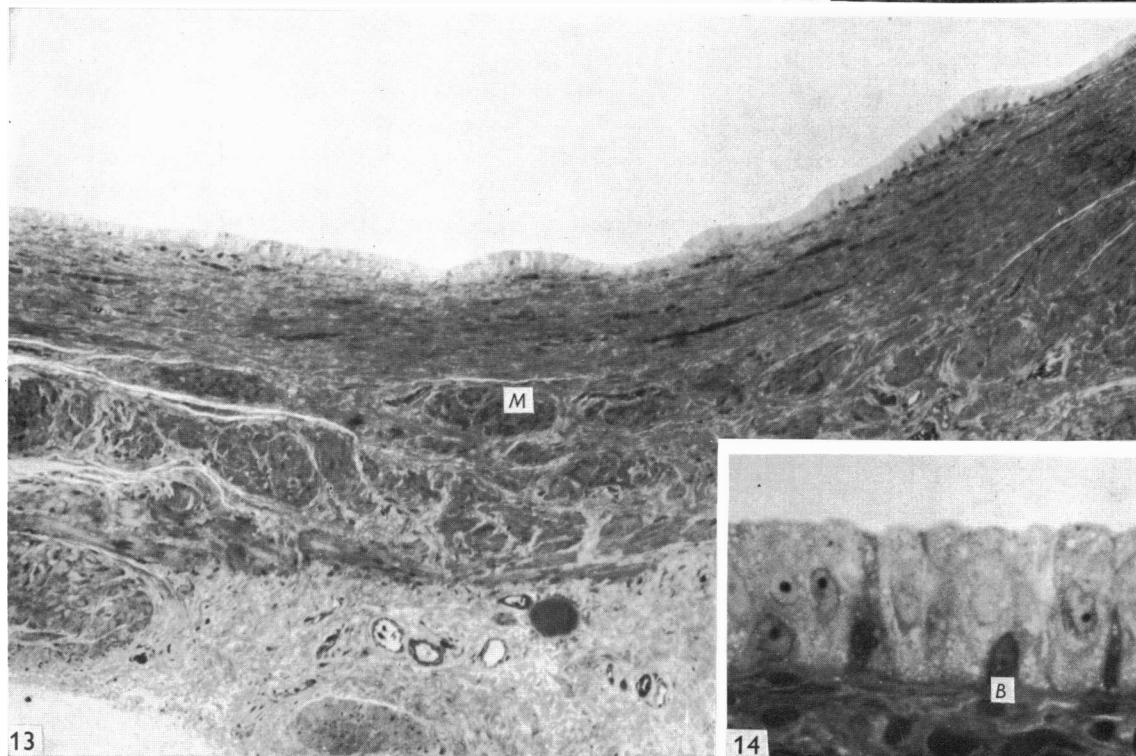
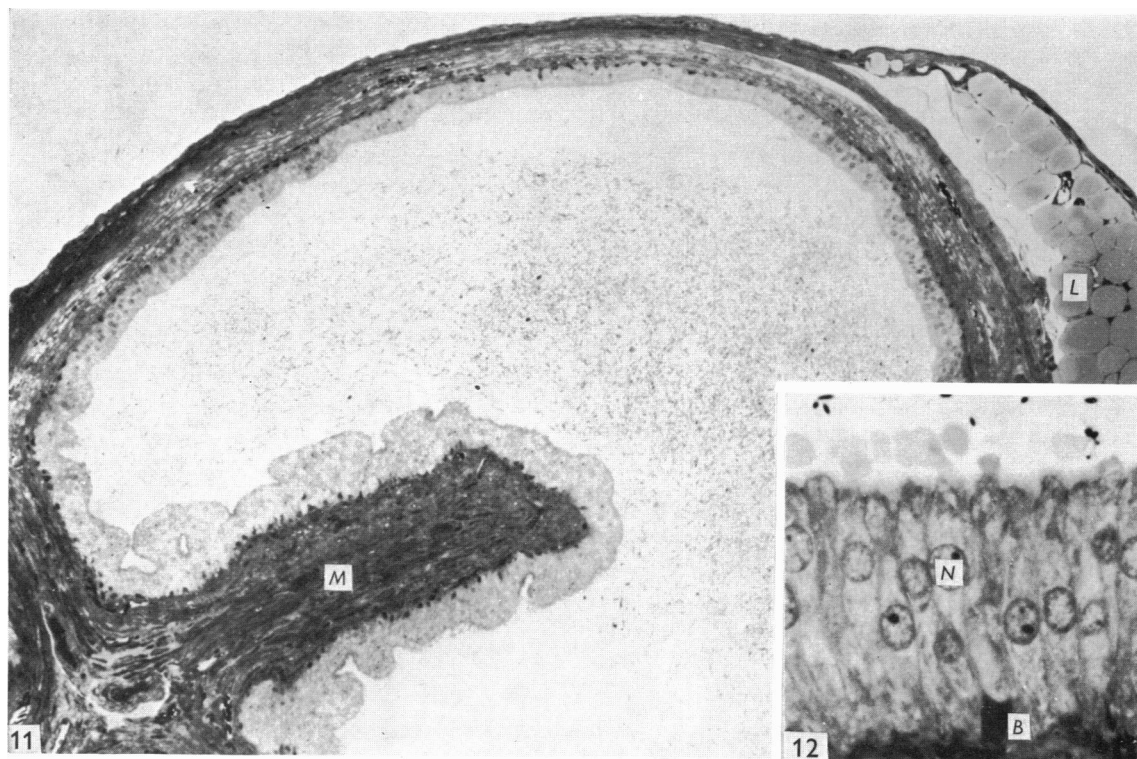
*Receptaculum ductus deferentis.* This sac-like region or dilation of the ductus deferens, which was difficult to dissect free of the fascia of the urodeum, had a wide lumen approximately 6 mm in diameter. A thick smooth muscle layer in fibrous tissue was found beneath the epithelium (Fig. 13), and a non-ciliated

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Fig. 8. The convoluted epithelium of the ductuli efferentes (*D*) and the relatively smooth epithelium of the ductus epididymidis (*E*) are compared here. Toluidine blue and basic fuchsin.  $\times 520$ .

Fig. 9. Ciliated cells of connecting ductules (*C*) are replaced by highly pseudostratified non-ciliated columnar cells (arrow) at the origin of the ductus epididymidis (*E*). Haematoxylin and eosin.  $\times 580$ .

Fig. 10. Non-ciliated columnar cells of the ductus epididymidis are characterized by cytoplasmic blebs (*C*) which extend into the ductal lumen. (*G*), granules; (*B*), basal cell. Toluidine blue and basic fuchsin.  $\times 1760$ .



pseudostratified columnar epithelium lined the lumen. Light and dark columnar cells were observed along with numerous dark basal cells (Fig. 14). The cells of the epithelium showed little evidence of blebbing or secretory activity.

*Papilla ductus deferentis.* The terminal portion of the ductus deferens, also reported as the 'ejaculatory duct' in the chicken (Lake, 1957; Tingari, 1971), opened into the urodeum through a small, round, erectile papilla. Outside the epithelial lining there was a thick layer of smooth muscle, and the lamina propria contained numerous blood sinuses (Fig. 15). The internal epithelium of the papilla was similar to that of the receptaculum being pseudostratified with light and dark columnar cells and basal cells (Fig. 16). The external surface of the papilla which was exposed to the lumen of the urodeum contained several crypts (Fig. 15) whose epithelial lining was pseudostratified with columnar cells. Goblet cells and cells with chromatophilic granules characterized this epithelium. Spermatozoa were frequently observed within the external crypts (Fig. 17).

#### DISCUSSION

The various parts of the avian reproductive tract are given different names in the literature. The chicken epididymis is made up of rete testis, ductuli efferentes and ductus epididymidis according to Kaupp (1915), Gray (1937), Parker, McKenzie & Kempster (1942) and Bradley & Grahame (1960). Lake (1957) referred to the region containing these structures as the 'epididymal region'. However, Tingari (1971) regarded each duct as a separate entity of the epididymal region. Lake (1957) and Tingari (1971) also referred to the terminal portion of the ductus deferens of the chicken as the 'dilation' which opens into the cloaca through the 'ejaculatory duct'. *Nomina Anatomica Avium* classified the dilated area as the 'receptaculum ductus deferentis' and the opening to the cloaca as the 'papilla ductus deferentis'. In this paper the nomenclature followed was that given in *Nomina Anatomica Avium*.

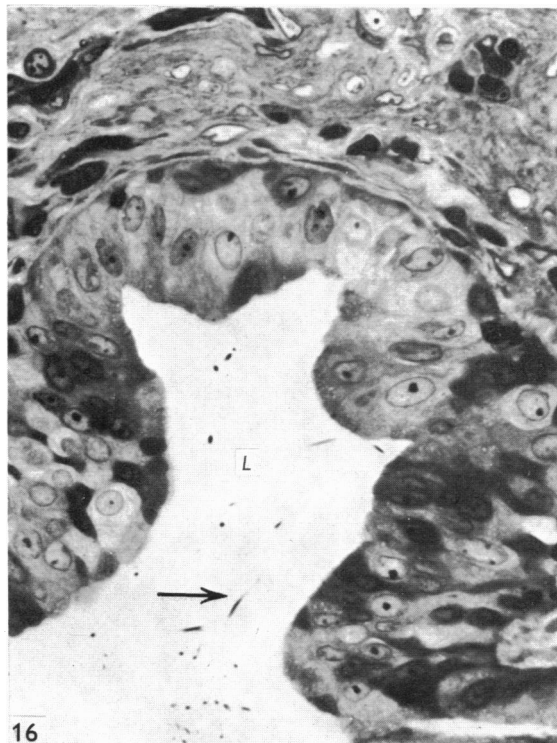
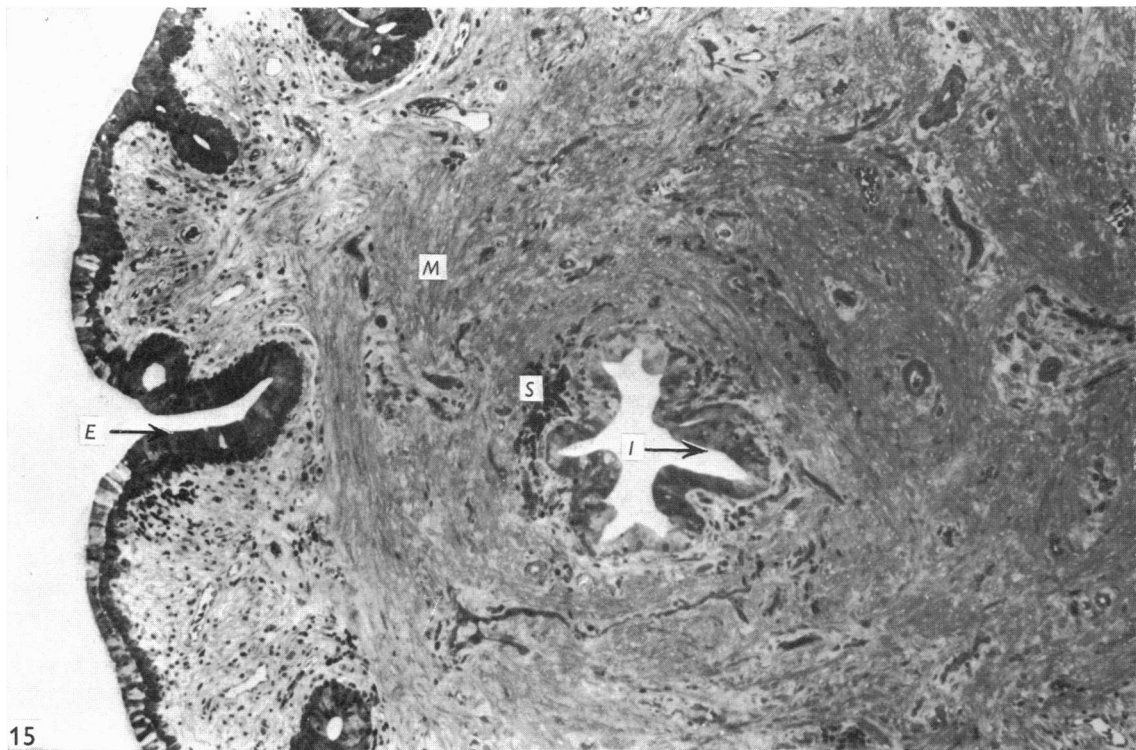
The gross anatomy of the male genital tract of the turkey, and the general succession of tubules in the epididymal region (rete testis, ductuli efferentes, connecting ductules, ductus epididymidis) were similar to those reported for the chicken (Stoll & Maraude, 1955; Lake, 1957; Tingari, 1971). The ductus epididymidis evidently occupies more of the epididymal region in the turkey than in the chicken since cross sections of this duct were seen not only at the periphery of the epididymal region, but also (unlike the chicken) next to the rete testis (Parker *et al.* 1942; Lake, 1957; Tingari, 1971).

The non-ciliated cells of the ductuli efferentes, ductus epididymidis and ductus deferens blebbed into the lumen of their respective ductules, which suggests apocrine secretion. If turkey spermatozoa are matured in the deferent duct as chicken spermatozoa are (Munro, 1938), then the secretory cells along the reproductive tract of the

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Figs. 11, 12. The pseudostratified columnar epithelium of the ductus deferens consists of non-ciliated columnar (N) and basal cells (B). (M), muscular layer; (L), lipid. Toluidine blue and basic fuchsin.  $\times 130$ .  $\times 830$ .

Figs. 13, 14. The receptaculum ductus deferentis has a thick layer of smooth muscle and muscle bundles (M). The pseudostratified columnar epithelium contains numerous basal cells (B). Toluidine blue and basic fuchsin.  $\times 135$ .  $\times 880$ .



turkey could contribute components of the seminal plasma necessary for sperm maturation. Since the turkey does not have accessory sex glands along its genital tract, it is reasonable to assume that the cells which bleb into the ductal lumen are genuinely secretory in nature rather than that the blebs are fixation artifacts. In this study the receptaculum and internal epithelium of the papilla did not contain secretory cells, suggesting that the primary function of the receptaculum is to store semen. The goblet cells of the external epithelium of the papilla possibly produce secretions which protect the papilla from the cloacal contents.

The non-ciliated cells of the ductuli efferentes and connecting ductules stained more intensely than the ciliated cells. This is opposite to the results reported by Tingari (1971) for equivalent cells in the chicken. Furthermore, Tingari (1971) reported that the epithelial linings of the chicken ductus epididymidis and ductus deferens contained light and dark staining cells. In the turkey only light staining cells were found (with haematoxylin and eosin stained, paraffin embedded tissue and toluidine blue and basic fuchsin stained Epon embedded tissue).

Aberrant ducts, like those reported in the chicken (Stoll & Maraude, 1955; Tingari, 1971) were not seen.

The sac-like receptaculum of the ductus deferens contained a large volume of semen at the time of dissection. Since this duct was surrounded by a thick muscle layer, it is probable that the receptaculum is the major contributor to the volume of semen at an ejaculation. Morphologically the receptaculum and papilla were similar to the ejaculatory ducts described by Lake (1957) in that they exhibited a thick fibromusculature, numerous blood sinuses for erectile activity and secretory cells in the external epithelium.

#### SUMMARY

The ductal system of the reproductive tract of the male domestic turkey was studied by gross dissection and light microscopy of paraffin and Epon embedded tissues. The succession of ductules as one passes caudally from the testis was as follows: seminiferous tubules; rete testis; ductuli efferentes; connecting ductules; ductus epididymidis; ductus deferens; receptaculum ductus deferentis; papilla ductus deferentis.

Non-ciliated cells of the male tract consisted of squamous and low cuboidal cells of the rete testis, granulated columnar cells lining the ductuli efferentes and connective ductules; agranulated columnar cells which formed the epithelium of the ductus epididymidis, ductus deferens, receptaculum and papilla ductus deferentis; and basal cells which were found in increasing number from the ductuli efferentes

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Fig. 15. The papilla ductus deferentis has an internal (*I*) and external (*E*) epithelium. Note the blood sinuses (*S*) and thick muscle layers (*M*). Toluidine blue and basic fuchsin.  $\times 135$ .

Fig. 16. A higher magnification of the internal epithelium of the papilla in Fig. 15. Light and dark staining columnar cells as well as basal cells are shown. (*L*), lumen; spermatozoon (arrow).  $\times 720$ .

Fig. 17. A crypt in the external, pseudostratified epithelium of a papilla contains goblet (*G*) and light staining columnar cells and many basal cells (*B*). Spermatozoa (arrow). Toluidine blue and basic fuchsin.  $\times 720$ .

to the papilla. The basal cells had a reduced amount of cytoplasm and stained more intensely than the other cell types. Ciliated cells were apparent in the ductuli efferentes and connecting ductules, and these consistently stained lighter than the non-ciliated cells. Non-ciliated columnar cells of the ductuli efferentes and connecting ductules contained chromatophilic granules. Cytoplasmic blebbing into the ductal lumina was found associated with these non-ciliated cells as well as the agranular cells of the ductus epididymidis and deferens. Evidence obtained from this study suggests that the non-ciliated cells of the ductuli efferentes, ductus epididymidis and ductus deferens have a contribution to make to the seminal plasma by apocrine secretion.

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